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THE SIZE OF THE MESH OPENINGS IN MUCOUS FEEDING NETS OF MARINE ANIMALS

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INTRODUCTION

In a former article (MacGinitie, 1937) the statement was made that "the slime net (Urechis) will entrap the smallest particles which are visible with the aid of an oil immersion lens." Experiments have just been completed which have determined much more accurately the size of the openings through which sea water is passed to strain from it the food ingested by animals using such nets. The results of these experiments have more than justified the statement quoted.

THE FEEDING NETS

The mucous net of the echiuroid worm *Urechis caupo* (Fisher and MacGinitie, 1928; MacGinitie, 1935) is admirably suited to tests of the kind herein reported. In the mud flats of the west coast Urechis builds a U-shaped tunnel with two entrances eighteen to thirty-six inches apart, depending on the size and age of the animal, and with the lowest part of the tunnel from ten to eighteen inches beneath the surface. The openings of the burrows are somewhat constricted, and the animals, which live singly in a burrow, keep a current of water flowing through the burrow along the body by means of a more or less rhythmic peristalsis.

The slime tube for collecting food is secreted by a ring of mucous glands situated about one-sixth of the length of the body posterior to the proboscis. When the worm secretes a mucous tube, this ring of glands is pressed tightly against the periphery of the burrow to cause the mucus at the beginning of secretion to adhere to the sides of the burrow. As the animal backs up in its burrow more mucus is spun until the tube becomes four to six inches long. Then the spinning ceases, but the tube is allowed to remain attached to the secreting ring. This leaves the animal with its anterior end within a sack of mucus which is firmly attached to the "neck" as though by a drawstring; and the distal end of the mucous sack is firmly attached to the sides of the burrow. Thus all water flowing through the burrow must pass in at the open distal end and pass through the walls of the mucous sack before it can flow along the outside of the animal on its way through the burrow. As the water passes through this mucous net all microscopic and, as will be seen, some ultramicroscopic, material is held by the net. After pumping water through the net for varying lengths of time, the end at the "neck" is slipped over the head and the entire tube with its load of food is swallowed.

The mucous food straining bag of the annelid *Chaetopterus variopedatus* (MacGinitie, 1939a) was also used for the purpose of testing size openings, though Chaetopterus is much more sensitive to strange substances than is Urechis. By using a liberal amount of time and patience, either animal becomes accustomed to the presence of proteins as they are introduced into the water currents passing through the burrows.

Materials and Method

Three substances of known molecular weight, and stained with T1824 Evan's Blue, were introduced into the mucous nets of both *Chaetopterus variopedatus* and *Urechis caupo* while the animals were feeding. The three substances and their molecular weights were: ovalbumin, 44,000; human serum globulin, 176,000; and hemocyanin (Palinurus), 450,000. The molecular weights given were taken from Cohn and Edsall (1943).

In the cases where excess dye was used for staining, the excess was taken up by dialyzing bags (Rawson, 1942–43), the proteins having been dissolved in sea water, 34 parts per thousand of salts. Rawson found that up to eight molecules of dye were bound by each molecule of globulin and that a 1 : 5 solution remained stable for one month. These figures were used also for ovalbumin and hemocyanin, though no experimental evidence was found for the use of eight molecules as a saturation limit, but absorption by cellophane dialzying bags showed these limits to be fairly accurate.

Results

The ovalbumin passed through the mucous net entirely. Enough serum globulin was held by the net to color it a decided blue, while no hemocyanin whatever passed through the mucus.

Molecular weights and shapes of proteins are subject to considerable uncertainty. Recently Cohn (1944) gives a molecular weight, length and equatorial diameter for human serum globulin of 160,000,320 Å, and 36 Å, respectively. The *a/b* ratio is thus about 1/9. Human serum globulin has a frictional flow coefficient of 1.49 and hemocyanin of 1.23. Comparison of the two molecules on this basis and disregarding hydration, gives hemocyanin an equatorial diameter of approximately 90Å, an *a/b* ratio of 1/5.

Ninety Ångströms seems to be a rather high figure, judging by what one can find in the literature relative to the diameters of protein molecules, but it is safe to say that the meshwork opening in a mucous feeding net is of a magnitude between 36 Å and 90Å. However, since some human serum globulin was caught by the net while no ovalbumin was, and because all hemocyanin was entrapped, a logical conclusion is that the meshwork openings are quite close to 40 Å.

Solutions varying from two molecules of dye to one of protein to those with an excess of dye were used with hemocyanin. Since the dye T1824 has a molecular weight of 960 (Rawson, 1942–43), the molecular weight of any of the substances used was not increased by more than 7,680. Human serum globulin was used in the proportion of five molecules of dye to one of the serum; thus the molecular weight was increased by 4,800. Allowing for this increase, the figure 40 Å was used instead of 36 Å, though the latter may be the more accurate figure.

The results described in this paper were the same for the mucous nets of both Chaetopterus and Urechis, and it is my opinion that all feeding nets where used by tunicates (MacGinitie, 1939b), pelecypods (MacGinitie, 1941), gastropods (in mss.), etc., are of like physical and chemical structure, though only actual tests with each animal will prove this to be true.

Discussion

There are several factors which preclude exact measurements, for example, uncertainty of molecular size, the shape of molecules, hydration, the shape of openings in the mucous network, surface conditions of both protein and mucoid molecules, rigidity of the mucus surrounding the openings, force or head of water flow, possible variation in the elasticity of the mucus, etc.

Molecular size should not show an error of more than plus or minus ten per cent or less, and the shape of molecules about the same or possibly more. It is difficult to conceive of straight openings from side to side of a mucous net .5 to 1 mm. in thickness. Where the mucous net remains attached to the body of Urechis it is about 3 mm. in thickness, and it was noted that the hemocyanin did not penetrate greatly beyond the inner surface. It did penetrate sufficiently, however, to become incorporated in the mucous network. It cannot be washed out, and later it is shown that no reaction takes place between the mucous tube and the hemocyanin. This indicates a sponge-like structure, and not openings running directly from side to side. Mehl (verbal communication) is of the opinion that the mucous molecules are long and slender. The last three factors, rigidity, force of flow and variation in elasticity, are more interdependent. The greater the rigidity, the more uniformly constant the openings, and the less affected by force of current.

Mucous nets are held under a certain amount of tension while the animal is feeding. When the net of Urechis is freed from the animal it at once collapses to one-half its original size and gradually draws up to much less than this if left free and immersed in sea water.

When Urechis is pumping water through a burrow of glass tubing for respiratory purposes (not feeding), and one end of the tube is held out of water the peristaltic movements can create a difference in head of four or more inches, which is equal to .144 lb. per square inch. Such pressures are never exerted upon a feeding tube. Under natural conditions perhaps but one-tenth of this amount of pressure is exerted upon the feeding tube.

A check was made to determine if on standing there was any reaction between the protein of the mucous tube and that of the substances used. Clean mucous nets were put in stained solutions of hemocyanin and human serum globulin and left for one-half hour periods, then centrifuged; no hemocyanin or serum globulin united with the tubes.

No dye has yet been found that will stain the pure mucus of a feeding net.

In one rechecking of these experiments, some hemocyanin (Palinurus) was used which had been precipitated by low pH and stored in a frozen state for about six months. This hemocyanin had denatured to the extent that it all passed through the mucous net. It is known that hemocyanin denatures to a greater extent than most proteins, though all hemocyanins are not alike in their amount of dissociation. Neurath, Greenstein, Putnam and Erickson (1944), quoting Burk (1940), give a dissociation for Limulus hemocyanin of from 2,040,000 to 142,000 for 6.66 M urea treatment and to 69,000 for HCl followed by 6.66 M urea. Measurements were made on the basis of osmotic pressures. This is a dissociation to about 1/14 for the urea treatment and 1/30 for the combined HCl-urea treatment. The difference between 142,000 and 69,000 is due to the removal of copper by the acid.

The fact that all of the hemocyanin used in this rechecking experiment passed through is proof that its molecular size had been reduced, at least in one dimension, by a low pH or by freezing, to a size lower than that of the human serum globulin.

Another factor, of which there are perhaps many more than were listed above, that could influence the passage of long molecules through a network such as that just described is the possibility that by physical means the molecules may be oriented in such a way that they may enter the network openings of the mucus small end first. This could be due to Brownian movement or electrical charge or flow influence. Perhaps these feeding nets may be of value in helping us to solve such problems.

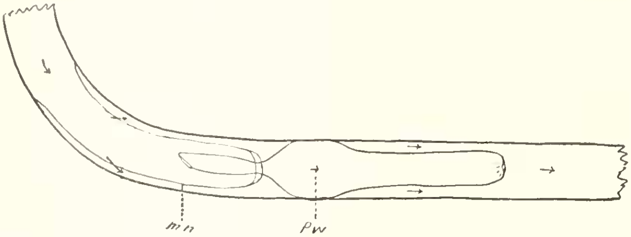

Diagram of *Urechis caupo* in a burrow, feeding. *m.n.*, mucous net; *p.w.*, peristaltic wave which drives water ahead of it. Other arrows indicate flow of water through the burrow.

The mucous net has been used to estimate the extent of polymerization in certain proteins. It already has been shown that it is a test for denaturation of hemocyanin (Palinurus). It will also be useful in checking partial dissociation or denaturation or for possible adulteration by smaller protein molecules.

There still remains the possibility that other animals may secrete mucous nets with meshwork different in porosity from that of Urechis and Chaetopterus, and further experimentation may show that the mucous net may be stretched or allowed to contract to further increase its range of usefulness.

Considering the above findings, one is impressed with the efficiency of mucus as a net for straining food from water. The molecular size of decomposition products would preclude their use as food material, but all other organic materials, including protein molecules of a size greater than 40 Å in cross section could be used.

It is interesting to think about a substance, which when it comes in contact with water, takes up 200 or more parts by weight of the latter, yet remains a meshwork which allows water to pass freely through it. It is also interesting that in its wide use among the marine invertebrates as a food gathering mechanism it is seldom if ever wasted, since it is necessarily swallowed with the food that it has entrapped.

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SUMMARY

1. A brief description is given of the food collecting net of the echiuroid worm *Urechis caupo*. Water flows freely through this mucous net.

2. By introducing into this mucous tube proteins of known molecular size, namely, ovalbumin, 44,000; human serum globulin, 176,000; and hemocyanin (Palinurus), 450,000, the size of the openings was found to be approximately 40 Ångströms.

3. Experimental uses for the mucous net are given, and other possible uses suggested.

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